Early steps of angiosperm–pollinator coevolution

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The hypothesis that early flowering plants were insect-pollinated could be tested by an examination of the pollination biology of basal angiosperms and the pollination modes of fossil angiosperms. We provide data to show that early fossil angiosperms were insect-pollinated. Eighty-six percent of 29 extant basal angiosperm families have species that are zoophilous (of which 34% are specialized) and 17% of the families have species that are wind-pollinated, whereas basal eudicot families and basal monocot families more commonly have wind and specialized pollination modes (up to 78%). Character reconstruction based on recent molecular trees of angiosperms suggests that the most parsimonious result is that zoophily is the ancestral state. Combining pollen ornamentation, size, and aperture characteristics and the abundance of single-species pollen clumps of Cenomanian angiosperm-dispersed pollen species from the Dakota Formation demonstrates a dominance of zoophilous pollination (76% versus 24% wind pollination). The zoophilous pollen species have adaptations for pollination by generalist insects (39%), specialized pollen-collecting insects (27%), and other specialized pollinators (10%). These data quantify the presences of more specialized pollination modes during the mid-Cretaceous angiosperm diversification.

Cretaceous | pollen | pollination biology | floral evolution

Early steps in angiosperm–pollinator coevolution are best understood through research on Cretaceous fossil flowers and *in situ* pollen (1–5). A widely accepted hypothesis is that insect pollination was the dominant mode of angiosperm pollination during the Early Cretaceous (4) with specialization increasing by the mid-Cretaceous (3). Even ancient relatives of extant wind-pollinated taxa have been suggested to be initially insect-pollinated, such as Late Albian flowers of *Platanus*-like plants (2) and Campanian fagaceous flowers (6). By the mid-Cretaceous, showy bisexual flowers indicate specialized insect pollination (3, 7, 8).

Understanding pollination biology of fossil plants is often based on morphological interpretations (3, 7, 9) using traits of fossil flowers and pollen compared with similar floral and pollen morphologies of living plants known to have specific pollinators. For example, pollen from wind-pollinated flowers appears to be dry with a smooth surface, of moderate size, and produced in large quantities (10–12). In contrast, zoophilous flowers appear to have pollen that is sticky with pollenkitt or other substances, generally with ornate surfaces, of variable size, and are produced in variable quantities (10, 13–16).

Other evidence central to a discussion of early angiosperm pollination biology is the fossil insect record (17–21) and pollinator modes of basal extant angiosperms (22, 23). Both support the zoophilous ancestral hypothesis (22, 23), with well preserved Early Cretaceous fossil insect pollinators (17–21) and insect pollination found in the most basal angiosperm families (Table 1). Yet pollination modes have not been analyzed phylogenetically for angiosperms.

We have taken two approaches that examine pollination systems in early angiosperms and their subsequent specialization. First, we looked at the phylogenetic distribution of pollination modes in extant basal angiosperms (dicots excluding the eudicots), basal monocots (families of Acorales and Alismatales), and basal eudicots (families of Ranunculales, Sabiales, Proteales, Trochodentrales, Buxales, and Gunnerales; refs. 24 and 25 and www.mobot.

Table 1. Comparison of pollination modes of basal angiosperm families (Table 2) and inferred modes for the Cenomanian pollen species (Table 3)

Plant group	No.	Pollination mode			
		Insect, %	Wind, %	Water, %	Specialized, %
Basal angiosperms	29	86	17	3	34
Basally placed monocots	15	40	27	40	75
Basally placed eudicots	16	56	63	0	78
Fossil monosulcate	16	87	13	NA	29
Fossil tricolpate	25	68	32	NA	65

Wind, insect, and water percentages are based on the total number of taxa in a group that have the mode divided by the number of taxa and thus can be counted more than once. Specialized percentages for the extant taxa are based on the total number of families with hymenoptera and water divided by the number of nonwind-pollinated families; specialized percentages for the fossils are based on morphology and frequency. NA, not available.

org/MOBOT/research/APweb; Table 2). This study allowed for the identification of the initial pollination mode and possible shifts in the pollination modes. Second, we examined dispersed angiosperm pollen grains and pollen clumps common in the mid-Cretaceous. Pollen clumps are found in zoophilous flowers in extant angiosperms (13, 15, 16), and this study provides evidence of extensive fossil pollen clumping. We suggest that this pollen-clumping character was a major step in angiosperm–pollinator coevolution. When such pollen clumps are found, they imply an increase in zoophilous pollination in the fossil record. We combine data on pollen clumping with data of pollen ornamentation, size, and aperture characters from single dispersed grains to provide frequencies of these characters as related to wind, general zoophilous, and specialized modes of pollination. Our data from the mid-Cretaceous (Middle Cenomanian) Dakota Formation (27) provides a test of early modes of pollination during a period of rapid angiosperm diversification (24, 28).

Results and Discussion

Although the pollination modes of extant basal angiosperms have been summarized (23, 29), these occurrences have not been compared with those found in basal eudicot and basal monocot families. Three groups of angiosperm families (refs. 24 and 25 and www. mobot.org/MOBOT/research/APweb) were examined: basal angiosperms (Table 2, Amborellaceae to Saururaceae), basal monocot (Table 2, Acoraceae to remaining monocots), and basal eudicots (Table 2, Berberidaceae to remaining eudicots). The pollinators were assigned to the following pollination modes based on Thien *et al.* (23): Coleoptera (beetle), Diptera (fly), Hymenoptera (mostly bee), Micropterigidae (basal family of Lepidoptera), Thysanoptera

Table 2. Extant angiosperm families and their pollination modes, divided by wind, animal, and type of pollinator if known

Family	Wind	Animal	Pollinator	Ref(s).
Amborellaceae	†	†	BDW	29
Nymphaeaceae		†	BDH	23,54,55
Cabombaceae		†	BDH	23,54
Austrobaileyaceae		†	BD?	23,56
Illiciaceae		†	BD	23,55
Schisandraceae		†	BD	23,54
Trimeniaceae	†	†	BHW	56
Ceratophyllaceae			Wa	23,55
Chloranthaceae	†	†	TW	23,54
Myristicaceae		†	BT	23,54
Magnoliaceae		†	BDTH	23,54,55
Degeneriaceae		†	B	23
Himantandraceae		†	Unknown	57
Eupomatiaceae		†	B	23,56
Annonaceae		†	BDT	23,54,55
Atherospermataceae			Unknown	
Calycanthaceae		†	B	23,56
Gomortegaceae		†	Unknown	56
Hernandiaceae		†	Unknown	56
Lauraceae		†	BDTH	23,55
Monimiaceae		†	BDTH	23,54
Siparunaceae			Unknown	
Canellaceae		†	BT	23
Winteraceae		†	BDMT	23,54,55
Aristolochiaceae		†	D	23,55
Hydnoraceae		†	B	56
Lactoridaceae	†		W	23
Piperaceae		†	BDH	23,55
Saururaceae	†	†	BDHTW	23
Acoraceae			Unknown	
Tofieldiaceae			Unknown	
Araceae		†	BDH	23,54
Alismataceae		†	DH	55
Aponogetonaceae		†	Unknown	56
Butomaceae		†	DH	58
Cymodoceaceae			Wa	56
Juncaginaceae	†		W	56
Hydrocharitaceae	†	†	BDWWa	55,59
Limnocharitaceae		†	Unknown	57
Posidoniaceae			Wa	56
Potamogetonaceae	†		WWa	55
Ruppiaceae			Wa	56
Scheuchzeriaceae	†		W	56
Zosteraceae			Wa	56
Remaining monocots	†	†	All	55
Berberidaceae		†	DH	55,60
Eupteleaceae	†		W	54,56
Circaeasteraceae		†	Unknown	57
Lardizabalaceae		†	DH	61
Menispermaceae	†	†	HW	54,55
Papaveraceae (inc. Fumarioideae Papaveroideae, Pteridophylloideae)		†	BDH	55
Ranunculaceae	†	†	HWO	54,55
Sabiaceae	†		W	54
Nelumbonaceae		†	BDH	54,62
Platanaceae	†		W	54,55
Proteaceae		†	BDH	26,54,55
Trochodendraceae	†	†	DW	54,56
Buxaceae	†		W	54
Didymelaceae	†		W	54
Gunneraceae	†		W	54
Myrothamnaceae	†		W	56
Remaining eudicots	†	†	All	55

† indicates presence; B, beetles (Coleoptera); D, Diptera (flies); H, Hymenoptera (mostly bees); M, Micropterigidae (basal family of Lepidoptera); T, thrips (Thysanoptera); W, wind; Wa, water; O, other.

(thrips), wind, water, other, or unknown (Table 2). Our data (Tables 1 and 2) show that 86% of the basal angiosperm families have species that are insect-pollinated, 17% of the families have wind-pollinated species, and 34% of the families with nonwind-pollinated species have specialized pollination modes, including water and Hymenoptera. In contrast, of the basal monocot families, only 40% have species that are insect-pollinated, 27% have wind-pollinated species, and 40% have water-pollinated species. However, specialized pollination is found in 75% of the basal monocot families with nonwind-pollinated species. Finally, in the basal eudicot families, 56% have insect-pollinated species (versus 63% wind) of which 78% have species with specialized pollination.

These pollination modes (Tables 1 and 2) are mapped on a mostly resolved conservative molecular tree (refs. 24 and 30–32 and www.mobot.org/MOBOT/research/Apweb; specifically figure 3.3 in ref. 24) and a fully resolved molecular tree (refs. 24, 30, 33, and 34; specifically figure 2.3 in ref. 24). As illustrated by the conservative tree (Fig. 1), using the most-parsimonious reconstruction (MPR) method (35), general insect pollination (most often beetles or flies) is initially present in all extant families of both the basal angiosperms (except water-pollinated Ceratophyllaceae and wind-pollinated Lactoridaceae), and the basal monocots. In contrast, the initial pollination mode for basal eudicots is equivocal, because of the increase of wind-pollinated clades (10 families; Table 2) early in eudicot evolution. With the MPR method, the resolved tree had the same result, whereas the delayed transformaton (DELTRAN) method also resolved the ancestral mode as insect pollination for the basal eudicots and the accelerated transformation (ACCTRAN) method likewise resolved the ancestral mode as wind. The families with wind and specialized pollination are clustered in the more derived families of basal monocots and basal eudicots, and these pollination modes appear to have evolved multiple times in these families in all analyses except for the eudicots in the ACCTRAN method. It is interesting to note that no bird, bat, or other more specialized pollination modes (10, 13) have been reported in these extant basal angiosperms and that finding is consistent with the appearance of bats and derived passerine birds during the Tertiary (36, 37). Molecular data suggest that early monocots originated and diverged between 147 million years ago (MYA) and 128 MYA, whereas the basal eudicot families originated and diverged between 125 MYA and 116 MYA (24). The fossil dates for the earliest evidence of each clade are 130 MYA and 125 MYA, respectively (38). Morphological, molecular, and fossil data show a similar Early Cretaceous age for the diversification of some insect pollinators (18). Thus, the distribution suggests increased wind and specialized pollination by the mid-Cretaceous and an increase of specialized pollination during the later Upper Cretaceous and Paleogene contemporaneously with continuing angiosperm radiations (1–7, 9).

We examined pollen from the mid-Cretaceous (Cenomanian) Dakota Formation in southwest Minnesota (27, 39) and found 41 angiosperm pollen species (Table 3) of which nine species (22%) were clumped (Fig. 2). These clumps range in size from 20 to >150 μm and include ≈5–800 pollen grains. Three types of pollen clumps are found (Table 3). (*i*) Four species have pollen clumps that are composed of many grains, either individual pollen grains or pollen tetrads, and also were commonly dispersed either as single grains or tetrads (frequencies of 23–72%). These include *Artiopollis indivisus* (Fig. 2 *A* and *B*), *Tricolpites* sp. (Fig. 2 *C* and *D*), cf. *Phimopollenites* (Fig. 2 *E* and *F*), and *Tricolpites* cf. *vulgaris* (Fig. 2*G*). (*ii*) Two species have pollen clumps composed of >50 individual grains, but have few dispersed pollen grains (frequencies of 0.2–0.7%). These include cf. *Psilatricolporites* sp. (Fig. 2*H*) and *Liliacidites* cf. *reticulatus* (Fig. 2*I*). (*iii*) Three species have pollen clumps each of which are composed of ≈10 individual grains that are also found commonly dispersed as individual pollen grains (frequencies of 7–21%). These include *Rousea* cf. *delicipollis* (Fig.
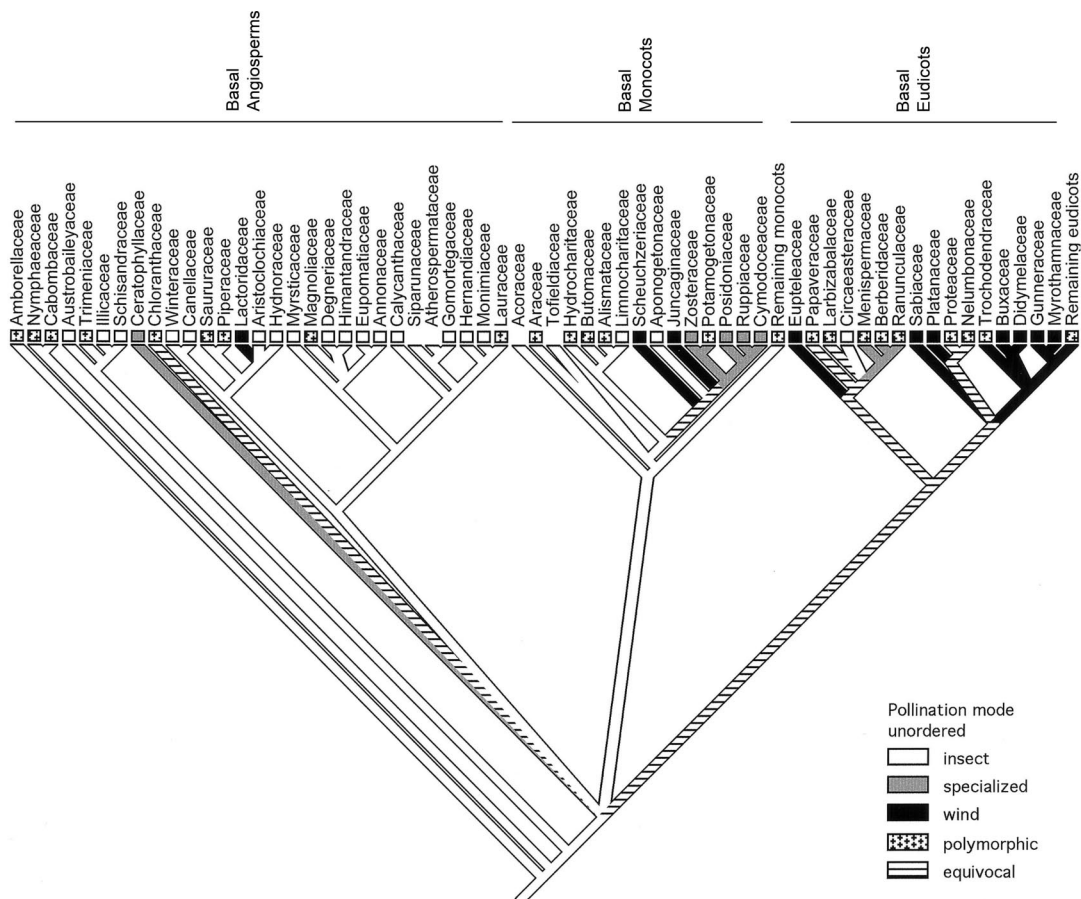
Fig. 1. MacClade reconstruction of the evolution of pollination modes based on molecular topology (refs. 24 and 30–32 and www.mobot.org/MOBOT/research/Apweb; specifically figure 3.3 in ref. 24). Missing rectangles by families indicate unknown pollination mode (Table 1). Insect pollination includes Coleoptera (beetle), Diptera (fly), and Thysanoptera (thrips). Specialized pollination includes water and Hymenoptera (mostly bee).

2*J*), *Cupuliferoidaepollenites* sp. (Fig. 2*K*), and *Dryadopollis* sp.1 (Fig. 2*L*).

Although two pollen species have been reported in clumps from the Early Cretaceous of Eastern North American (40, 41), the importance of clumping was not appreciated until recently (11, 15, 16). Clumping may be caused by several methods including viscous fluids (tryphine, pollenkitt, elastoviscin), tangling, and common walls, resulting in clumps of variable sizes or some with uniform sizes and numbers of grains in permanent polyads and pollinia (15). Clusters of more than a few grains are rare in wind-pollinated plants because clumped pollen falls faster (11, 15, 16). The sticky surface of the pollen that causes clumping plays several roles in pollen dispersal and pollination, including retaining pollen in the anther, increasing adhesion to the pollinator, and transporting multiple pollen grains to the stigma (15). Multiple pollen grains on the stigma appear to increase fitness through pollen tube competition (42), are advantageous when pollinators are in low numbers or nonspecific in behavior (43), and lower the chance of individual pollen grains becoming dehydrated (44). Because of the important role pollen clumps can play in the reproductive biology of animal-pollinated plants, their occurrence is a significant step in early flowering plant evolution (11, 15, 16).

Clumped fossil pollen may occur naturally or result from the remains of anthers, insect pellets, and insect packaging (40, 45, 46). Cretaceous pollen clumps have been suggested to be the contents of immature fossil anther fragments included in the preparation of dispersed grains (40). Our data show that clumped and dispersed grains of the same type have the same size and ornamentation and are not arranged in immature tetrads (Fig. 2), and each specific pollen type may be in clumps that are of variable sizes and shapes. In addition, no flower or anther mesofossils were found, although other mesofossils were discovered at the same localities (27, 39), suggesting that the clumps contain mature grains and are not derived from fragmented immature anthers. Pollen clumps in insect pellets have also been reported as products of insect activity (45, 46). However, the possibility that the pollen clumps reported here are from fecal pellets seems unlikely as the pollen grains in the fossil clumps are complete and show no signs of damage originating from insect chewing and digestion, and the entire clumps are not regular in shape with smooth margins as would be expected in fecal pellets. Moreover, fecal pellet mesofossils were found at the same localities, but these did not contain pollen grains (S.H., personal observation). Modern bees often make pollen packages, although they usually have a mix of pollen types (47). None of the fossil clumps have more than one pollen type, so insect packaging is unlikely.

The fossil clumps are likely caused by pollen stickiness and originated either from mature fossil anthers or as dispersal events. In either case, the stickiness that forms the pollen clumps indicates an adaptation for zoophilous pollination. Additionally, none of the characters associated with wind pollination (including being dry when dispersed) were found in the fossil clumps (Table 3). Finally, clumping has been observed in fossil flowers. One of these fossil flowers shows pollen grains clustered in the anthers and clumps of identical pollen on the stigma (9). There is strong evidence that these flowers were pollinated by bees (9, 48). We propose that fossil pollen clumping is evidence for sticky pollen and that this character evolved by the mid-Cretaceous.
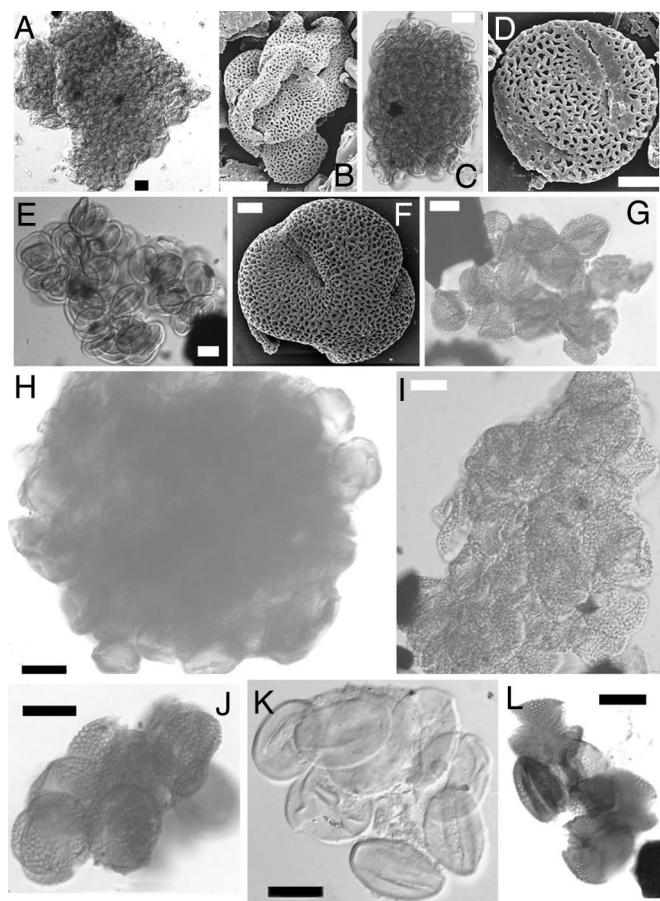
Table 3. List of pollen taxa identified in the Cenomanian Dakota Formation, Minnesota (27)

Pollen type	Ornamentation	Grain size, μm	Aperture type	Clumps Size, μm	Clumps No. of grains	Clumps No.	C	H	O	Pollination mode
Clavatipollenites tenellis	Reticulate	28	Monosulcate	NA	NA	NA	P	A	0.1	Zoo. gen.
Clavatipollenites sp.2	Reticulate	24	Monosulcate	NA	NA	NA	P	A	0.2	Zoo. gen.
?*Clavatipollenites* sp.3	Microfoveolate	27	Monosulcate	NA	NA	NA	A	A	0.9	Wind (?)
Liliacidites giganteus	Reticulate	76	Monosulcate	NA	NA	NA	P	A	A	Zoo. spec. oth.
Liliacidites cf. *inaequalis*	Reticulate	24	Monosulcate	NA	NA	NA	A	1.0	A	Zoo. gen.
Liliacidites cf. *reticulatus*	Reticulate	24	Monosulcate	110 × 64	50	1, type 2	P	0.7	P	Zoo. gen.
Liliacidites sp.1	Reticulate	34	Monosulcate	NA	NA	NA	A	A	0.9	Zoo. gen.
Liliacidites sp.2	Reticulate	21	Monosulcate	NA	NA	NA	A	A	P	Zoo. spec. oth.
Liliacidites sp.3	Reticulate	30	Monosulcate	NA	NA	NA	A	A	3.0	Zoo. gen.
Liliacidites sp.4	Reticulate	37	Monosulcate	NA	NA	NA	A	A	0.7	Zoo. gen.
Liliacidites sp.5	Reticulate	24	Monosulcate	NA	NA	NA	A	0.3	P	Zoo. gen.
Retimonocolpites dividuus	Reticulate	35	Monosulcate	NA	NA	NA	P	A	0.7	Zoo. gen.
? *Spinizonocolpites* sp.	Scabrate	25	Monosulcate	NA	NA	NA	P	A	A	Wind (?)
Stellatopollis largissimus	Reticulate	123	Monosulcate	NA	NA	NA	P	A	A	Zoo. spec. oth.
Stellatopollis sp.	Reticulate	53	Monosulcate	NA	NA	NA	P	A	A	Zoo. spec. oth.
Doyleipollenites robbinsiae	Reticulate to foveolate	27	Trichotomosulcate	NA	NA	NA	0.5	A	0.5	Zoo. gen.
Artiopollis indivisus (tetrad)	Microreticulate	13	Tricolpate	36 × 33–151 × 142	24–800	3, type 1	A	A	30	Zoo. spec. p. c.
Cupuliferoidaepollenites sp.	Scabrate	16	Tricolpate	47 × 38–35 × 26	8–9	2, type 3	21	A	9.0	Zoo. spec. p. c.
Foveotricolpites sp.	Foveolate	38	Tricolpate	NA	NA	NA	A	A	0.4	Wind
Fraxinoipollenites constrictus	Microfoveolate	38	Tricolpate	NA	NA	NA	5.0	5.0	0.9	Wind
Rousea cf. *delicipollis*	Reticulate to foveolate	23	Tricolpate	52 × 30–46 × 31	10	2, type 3	7.0	P	A	Zoo. spec. p. c.
Satishia sp.	Microreticulate	28	Tricolpate	NA	NA	NA	P	A	A	Wind (?)
Striatopollis paraneus	Striato-reticulate	21	Tricolpate	NA	NA	NA	2.0	A	A	Zoo. spec. p. c.
Tricolpites sp.	Reticulate	14	Tricolpate	27 × 19–76 × 62	10–200	7, type 1	13	A	23	Zoo. spec. p. c.
Tricolpites nemejci	Microreticulate	27	Tricolpate	NA	NA	NA	0.3	A	3.0	Wind (?)
Tricolpites cf. *vulgaris*	Reticulate	20	Tricolpate	37 × 23–82 × 70	5–100	5, type 1	39	21	12	Zoo. spec. p. c.
Tricolpate sp.4	Reticulate	22	Tricolpate	NA	NA	NA	4.0	A	4.0	Zoo. spec. p. c.
Tricolpate sp.7	Microfoveolate	18	Tricolpate	NA	NA	NA	P	A	0.5	Zoo. gen.
Tricolpate sp.8	Scabrate	26	Tricolpate	NA	NA	NA	A	A	0.2	Wind (?)
Tricolpate sp.10	Microreticulate to microfoveolate	29	Tricolpate	NA	NA	NA	A	A	1.0	Wind
Tricolpate sp.11	Reticulate to foveolate	15	Tricolpate	NA	NA	NA	P	A	0.5	Zoo. gen.
Tricolpate sp.12	Reticulate	19	Tricolpate	NA	NA	NA	A	A	3.0	Zoo. spec. p. c.
Tricolpate sp.14	Microfoveolate	18	Tricolpate	NA	NA	NA	A	A	0.9	Zoo. gen.
cf. *Phimopollenites* sp.	Microreticulate	19	Tricolporoidate	20 × 13–107 × 75	7–50	3, type 1	P	72	2.0	Zoo. spec. p. c.
cf. *Psilatricolporites* sp.	Scabrate	14	Tricolporoidate	91 × 88	100	1, type 2	A	A	0.2	Zoo. gen.
Dryadopollis sp.1	Microreticulate	19	Tricolporate	66 × 38–62 × 30	7–15	2, type 3	7.0	A	A	Zoo. spec. p. c.
Dryadopollis sp.2	Microreticulate	10	Tricolporate	NA	NA	NA	0.3	A	A	Zoo. gen.
Foveotricolporites rhombohedralis	Microfoveolate	47	Tricolporate	NA	NA	NA	0.5	A	0.5	Zoo. gen.
cf. *Foveotricolporites* sp.	Microfoveolate	30	Tricolporate	NA	NA	NA	P	A	A	Wind (?)
Nyssapollenites sp.	Microfoveolate	14	Tricolporate	NA	NA	NA	0.8	A	2.0	Zoo. spec. p. c.
Tricolporate sp.2	Striate	25	Tricolporate	NA	NA	NA	A	A	P	Wind (?)

Monosulcate pollen grains are listed first followed by tricolpate and tricolporate. Grain size is average of largest dimension. Frequencies were calculated based only on the dispersed grains found (nonangiospermous taxa were excluded) at Courtland Clay Pit (C), Highway 4 Clay Pit (H), Ochs Clay Pit (O). Present (P) indicates species found but not in random counts of grains used to calculate frequencies. A, absent. Modes of pollination are based on Table 4: Zoo., zoophilous; gen., general; spec., specialized; p. c., pollen collecting; oth., other; NA, not available.

We define here four modes of pollination: wind, zoophilous general, zoophilous-specialized pollen collecting, and zoophilous-specialized other, based on literature about extant plants (Table 4). Pollen from wind-pollinated species has smooth ornamentation (e.g., scabrate, microreticulate, microfoveolate, striate), a dry surface, a size range of 25 to 40 μm, with little (two to three grains) or no clumping, and pollen produced in large quantities (10–12). Zoophilous-general flowers have grains that are ornamented, may be sticky, are 10–300 μm in size, can be clumped, and are produced at variable numbers (4, 10, 13, 14). Pollen grains from zoophilous-specialized pollen-collecting flowers are usually clumped, <24 μm in size, and produced at high numbers (13, 49, 50). Pollen grains classified as zoophilous-specialized other are usually of large size and produced in low numbers (14, 49, 50).

Accordingly, each of the 41 fossil species of clumped and dispersed pollen is assigned to one of these four suggested modes

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Fig. 2. Pollen species from the Cenomanian Dakota Formation, Minnesota (27, 39) that shows clumping. (*A* and *B*) *Artiopollis indivisus* Agasie, 1969. (*A*) 046535-PY02A, S32, midfocus. Pollen clump. (*B*) SEM, 046533 stub3. Individual dispersed grain. (*C* and *D*) *Tricolpites* sp. (*C*) 046533-PY03A, R35/1, midfocus. Pollen clump. (*D*) SEM, 046533 stub3. Individual dispersed grain. (*E* and *F*) cf. *Phimopollenites* sp. (*E*) 046517-A1, + 10 μm, F14, midfocus. Pollen clump. (*F*) SEM, 046517 stub 1, polar view. Individual dispersed grain. (*G*) *Tricolpites* cf. *vulgaris* (Pierce) Srivastava, 1969, 036690 + 10 μm, Y42/2, midfocus. Pollen clump. (*H*) cf. *Psilatricolporites* sp., 046522-PY01A, N29/3, midfocus. Pollen clump. (*I*) *Liliacidites* cf. *reticulatus* (Brenner) Singh, 1971, 036716-A5 + 10 μm, M16, midfocus. Pollen clump. (*J*) *Rousea* cf. *delicipollis* Srivastava, 1975, 18297 + 10 μm, V33/2, midfocus. Pollen clump. (*K*) *Cupuliferoidaepollenites* sp., 046522-PY01A, Q18/4, midfocus. Pollen clump. (*L*) *Dryadopollis* sp.1, 036708 + 10 μm, N36, midfocus. Pollen clump. (Scale bars: *A*, *C*, *E*, and *G–L*, 10 μm; *B*, 5 μm; *D* and *F*, 2 μm.)

of grains is probably not the best trait for distinguishing the wind mode from zoophilous modes in the fossil record. The remaining 31 species are classified as zoophilous based on ornamentation and size and are divided into three modes. Sixteen fossil pollen species have the traits of the general zoophilous pollination mode including low frequencies (0.1–3%, generally lower than the wind-pollinated species) and variable size (39% of the dispersed species, two species with type 2 clumps; Table 3). Nevertheless, the fact that two of these species have clumped pollen indicates advances in the evolution of sticky pollen. Eleven fossil pollen species that occur in frequencies from 2–72% and are <24 μm in size (27% of the dispersed species, seven species with type 1 or 3 clumps; Table 3) are typical of pollen grains produced for the zoophilous-specialized pollen-collecting mode. In these cases stickiness is important for pollinator transport and the majority of our fossil species were found as type 1 or 3 clumps (Table 3). High pollen production is typical in modern insect-pollinated angiosperms that use pollen as a primary attractant (13), with flowers adapted for bee pollination known to produce large quantities of pollen (51). There is fossil evidence of pollen feeders from a mid-Cretaceous flower that produced large quantities of pollen (46). Finally, four fossil species that are extremely rare and usually have large pollen grains (10% of the dispersed species) are similar to those with zoophilous-specialized other modes. Large pollen grains are found in some derived lepidopterian pollination systems (49).

The diversity including generalized to specialized insect pollination observed in extant angiosperms is also found in the fossil pollen species reported here (Table 1). Monosulcate pollen is found in extant basal angiosperms and monocots, and we infer that 87% of the fossil pollen species reported here were insect-pollinated and that 29% of the fossil pollen species had specialized modes of pollination (Table 1), mostly in the monosulcate (putatively monocot) species (Table 3). This finding is in contrast to fossil tricolpate-derived species (putatively eudicots) of which 65% are inferred to have specialized modes (Tables 1 and 3). The number of wind-pollinated species also increases from 13% in fossil monosulcate to 32% in fossil tricolpate species (Table 1).

During the Early Cretaceous, angiosperm pollen production was low (4), and apparently clumping was rare (40). Our data strongly suggest that by the mid-Cretaceous there is evidence of adaptations to permit pollen clumping and increases in specialized pollinators, which is consistent with insect molecular phylogeny showing bees originated between 110 MYA and 90 MYA (18), and the earliest fossil bee is reported from Early Cretaceous (21). This is a period of major radiation for the angiosperms (24, 28). Thus, the increase in specialized pollination modes may be linked to bee pollination.

Our reconstruction of the evolution of pollination modes supports the hypothesis that insect pollination is the initial pollination mode for angiosperms and suggests that more specialized animal pollination modes are derived. Fossil data from the mid-Cretaceous pollen record also provide evidence for specialized pollination modes and indicates that pollenkitt and other compounds that permit pollen clumping appeared later. Together, the pollination modes of extant basal angiosperms, coupled with dispersed and clumped pollen data, support the hypothesis that zoophilous pollination was common during the mid-Cretaceous and that special-

of pollination. The first group of fossil pollen has characteristics of the wind mode including "wind?" in Table 3, designated for grains with wind morphology but low abundance, includes 24% of species, is always found dispersed, and is never in clumps. Ten species in this wind mode type range from being rare to having frequencies up to 5%. Based on the variation of abundance between sites, frequency

Table 4. Criteria for wind and zoophilous modes of pollination based on extant plants

Pollination mode	Surface feature	Pollen size, μm	Dispersal method	Pollen production
Wind-pollinated	Smooth and dry	25–40	Individually	Large quantities
Animal-pollinated				
Zoophilous general	Ornamental, may be sticky and oily	10–300	Individually or clumped	Variable quantities
Zoophilous specialized pollen collecting	Ornamental, sticky, and oily	<24 (?)	Usually clumped	Large quantities
Zoophilous specialized other	Ornamental, sticky, and oily	Usually large	Individually, clumped (?)	Low quantities

ization had begun to occur. These hypotheses on the evolution of specific modes of pollination and pollen stickiness need to be tested further with studies specifically looking for angiosperm fossil pollen clumping during the Early Cretaceous.

Materials and Methods

Three groups of angiosperm families (refs. 24 and 25, www.mobot.org/MOBOT/research/Apwe, and Table 2) were examined: noneudicots and nonmonocots (Amborellales, Nymphaeales, Austrobaileyales, Ceratophyllales, Chloranthales, Magnoliales, Laurales, Canellales, Piperales), basal monocot clades (Acorales, Alismatales), and basal eudicot clades (Ranunculales, Sabiales, Proteales, Trochodendrales, Buxales, Gunnerales). Pollinators were identified for the species of each family and placed in the following pollinator groups: Coleoptera (beetle), Diptera, Hymenoptera, Micropterigidae (basal family of Lepidoptera), Thysanoptera (thrips), wind, and water (refs. 23 and 29 and Table 2). Data were summarized as follows. First, pollination modes (52) for the species from each family were identified as wind, insect, or water. Families with more than one mode were counted multiple times. Percentages were based on the number of families with the mode divided by the total number of families in the group; thus, many families were counted more than once. Second, the number of families with specialized pollination (water and Hymenoptera) were counted, and percentages were calculated by dividing by the number of nonwind-pollinated families.

The MacClade (35) reconstruction of the pollination modes was based on two molecular trees, a conservative unresolved tree (refs. 24 and 30–32 and www.mobot.org/MOBOT/research/Apweb; specifically figure 3.3 in ref. 24, shown in Fig. 1), and a less-supported resolved tree (refs. 24, 30, 33, and 34, specifically figure 2.3 in ref. 24 and data not shown). The states were considered unordered and reversible, and polymorphic families were coded for all possible pollinator states. The unresolved tree could only be analyzed by the MPR method, whereas the resolved trees were also analyzed by DELTRAN and ACCTRAN. The definition of insect and specialized states is as above.

Three localities of the Cenomanian Dakota Formation in southwest Minnesota (27, 39) were investigated: Courtland Clay Pit (latitude 44°16′29″ N, longitude 94°23′13″W), Highway 4 Clay Pit (latitude 44°26′05″ N, longitude 94°43′37″W), and Ochs Clay Pit (latitude 44°13′26″ N, longitude 95°00′42″W). Pollen samples were collected vertically at 30-cm intervals from each of the sections sampled. Abundant palynomorphs where found in eight samples at the Courtland Clay Pit, three samples at Highway 4 Clay Pit, and seven samples at Ochs Clay Pit.

These samples were processed at the Paleobotany and Palynology Laboratory of the Florida Museum of Natural History, using standard pollen processing methods for siliciclastic and lignite samples (27, 53). All samples were coarsely crushed and sieved with a tea strainer before chemical processing. At least two slides were scanned to find pollen clumps and build a catalog of pollen and spore types for each sample. At least 300 palynomorphs were randomly counted to calculate frequencies. Frequencies in Table 3 were calculated based on dispersed grains identified, excluding nonangiospermous taxa. A Zeiss Axiophot microscope and an AxioCam digital camera and imaging capturing software were used for palynomorph identification and photography. Slides are stored in the Paleobotany and Palynology Collection of the Florida Museum of Natural History.

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